THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplementary Information

In-house PCR for 2019 novel coronavirus detection

Extraction of viral nucleic acid from respiratory specimens was performed using EZ1 virus mini kit v2.0 (Qiagen) according to manufacturer's instruction. The RNA was eluted in 60μl of AVE buffer and used as template for all assays.

Two specific real-time RT-PCR methods targeting the *N* and *ORF1ab*, were designed to detect the presence of 2019-nCoV in clinical samples. The *N* gene primer sequences are: forward primer 5' CTC AGT CCA AGA TGG TAT TTC T; reverse primer 5' AGC ACC ATA GGG AAG TCC. The probe sequence is: 5' FAM-ACC TAG GAA CTG GCC CAG AAG CT-BHQ1, as previously described. Thermal cycling was performed at $50^{\circ}C$ for 20 min for reverse transcription, followed by $95^{\circ}C$ for 15 min and then 50 cycles of $94^{\circ}C$ for 5 s, $55^{\circ}C$ for 1min.

The sequence for the *ORF1ab* real-time RT-PCR are: forward primer 5' TCA TTG TTA ATG CCT ATA TTA ACC; reverse primer: 5' CAC TTA ATG TAA GGC TTT GTT AAG; probe: 5' FAM- AAC TGC AGA GTC ACA TGT TGA CA-BHQ1. Thermal cycling for both *ORF1ab* was performed at $50^{\circ}C$ for 20 min for reverse transcription, followed by $95^{\circ}C$ for 15 min and then 50 cycles of $94^{\circ}C$ for 5 s, $50^{\circ}C$ for 20s and $72^{\circ}C$ for 20s.

For both assays, a 20μl reaction containing 5μl RNA template, 500nm each of forward and reverse primer, 150nm probe and 0.2μl QuantiTect RT mix was prepared using QuantiTect Probe RT-PCR kit (Qiagen). All reactions were run on LightCycler 2.0 instrument (Roche).

All samples were also tested for endogenous RNAse P as an internal control.

Phylogenetic Analysis

Viral genomic sequences were available for 4 cases described in cluster A. Household cases AH1, AH2 and AH3 share identical sequences over the full genome length and differ by only 2 base differences from linked case AT1 as well as from early outbreak consensus sequences from Wuhan. All four linked cases in cluster A share a common base difference relative to the earlier sequences. The probability to have a shared base difference by chance is 3e-05 for any pair and 1e-18 for four sequences. Therefore, the genetic linkage of the 4 cases strongly supports the available epidemiological data.
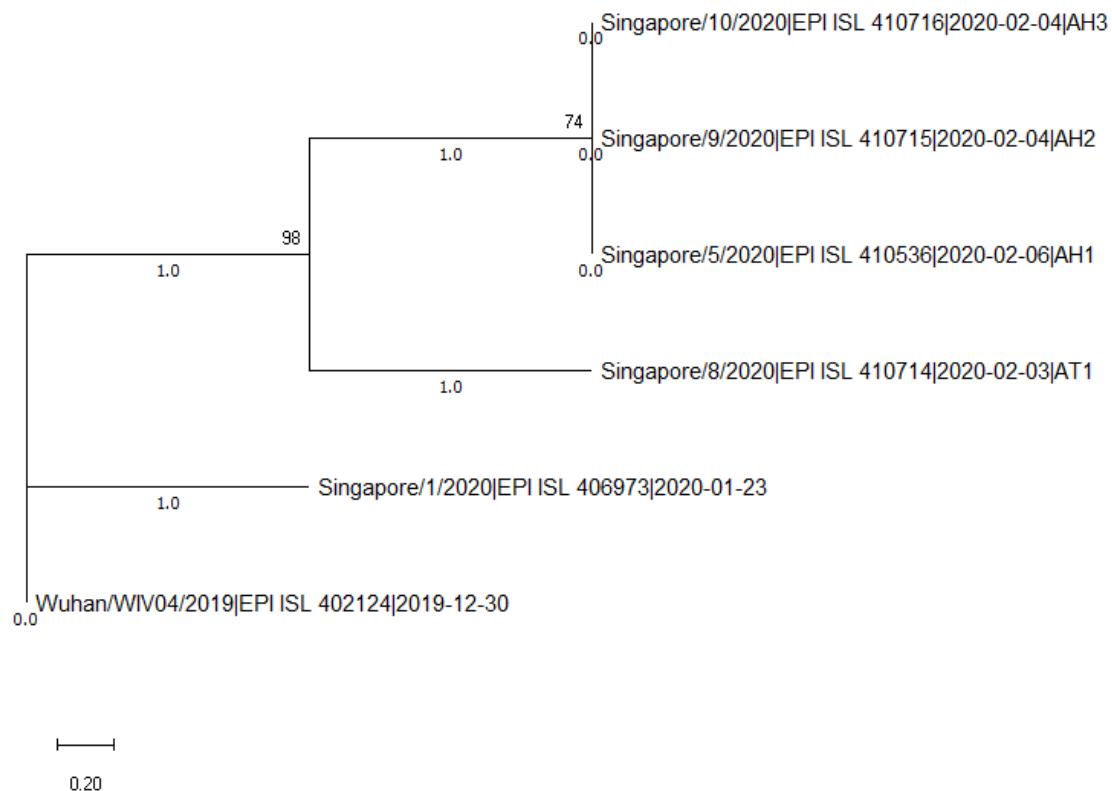

Figure Legend: Phylogenetic full genome tree of cases from cluster A with early outbreak sequence from Wuhan and the first detected case in Singapore. The branch lengths are drawn to scale in the units of the number of base differences per sequence shown below the branches.

Methods: Phylogenetic tree using the Neighbor-Joining method [1] and confirmed to be consistent using Maximum Likelihood approaches (not shown). The percentage of replicate trees in which the associated taxa clustered together in the 500 replicates bootstrap test are shown next to the subtree junctions [2]. The tree is drawn to scale, with branch lengths in the units of the number of base differences per sequence [3] shown below the branches. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 29891 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [4]. Strain names, GISAID EpiCoV accession numbers and collection dates are shown, followed by the case number if available. We gratefully acknowledge the Authors, the Originating and Submitting Laboratories for their sequence and metadata shared through GISAID, on which this research is based. The full acknowledgements are given in Supplementary Table S2. It should be noted that Singapore/5 has been obtained from a cell

passaged isolate while all the others are from direct sequencing of clinical samples. Since Singapore/5 is part of the household cluster with identical genomes, we believe cell passage adaptation does not constitute a confounding factor for this analysis.

1. Saitou N. and Nei M. (**1987**). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425.
2. Felsenstein J. (**1985**). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791.
3. Nei M. and Kumar S. (**2000**). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
4. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (**2018**). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**:1547-1549.

Table S1: Case definitions used in Singapore.

Date	Suspect case definition
2 January 2020 (Initial)	(a) A person with clinical signs and symptoms suggestive of <u>pneumonia</u> or <u>severe respiratory infection with breathlessness</u> **AND** travel to or residence in **Wuhan city** within the last 14 days; or (b) A person with an <u>acute respiratory illness of any degree of severity</u> who, within 14 days before onset of illness, had **close contact**[1] with a pneumonia case of unknown cause linked to the Wuhan cluster.
21 January 2020	(a) A person with clinical signs and symptoms suggestive of <u>pneumonia</u> or <u>severe respiratory infection with breathlessness</u> AND travel to **mainland China** within 14 days before onset of illness; or (b) A person with an <u>acute respiratory illness of any degree of severity</u> who, within 14 days before onset of illness, had **been to a hospital in mainland China** or had close contact[1] with a case of 2019 novel coronavirus infection.
25 January 2020	(a) A person with clinical signs and symptoms suggestive of <u>pneumonia</u> or <u>severe respiratory infection with breathlessness</u> AND travel to mainland China within 14 days before onset of illness; or (b) A person with an <u>acute respiratory illness of any degree of severity</u> who, within 14 days before onset of illness had: a. Been to **Wuhan city or Hubei Province, China**; OR b. Been to a hospital in mainland China; OR c. Had close contact[1] with a case of 2019 novel coronavirus infection.
4 February 2020 (current)	(a) A person with clinical signs and symptoms suggestive of <u>pneumonia</u> or <u>severe respiratory infection with breathlessness</u> AND travel to mainland China within 14 days before onset of illness; or (b) A person with an <u>acute respiratory illness of any degree of severity</u> who, within 14 days before onset of illness had: a. Been to **Hubei Province (including Wuhan city) or Zhejiang Province (including Hangzhou city)**, China; OR b. Been to a hospital in mainland China; OR c. Had close contact[1] with a case of 2019 novel coronavirus infection; OR d. Had **frequent or close contact during work**[2] **with recent travellers from mainland China** (travel history in the last 14 days).

[1]Close contact is defined as: Anyone who provided care for the patient, including a health care worker or family member, or who had other similarly close physical contact; Anyone who stayed (e.g. lived with, visited) at the same place as a case.

[2]Persons who attended business meetings/discussions, frontline staff in hospitality and tourism (e.g. hotels, shops, tours) with regular dealings with travellers from mainland China.

Table S2: Authors, originating and submitting laboratories of sequences used in phylogenetic analysis.

We gratefully acknowledge the Authors, the Originating and Submitting Laboratories for their sequence and metadata shared through GISAID, on which this research is based. All submitters of data may be contacted directly via www.gisaid.org.

Accession ID	Virus name	Location	Collection date	Originating lab	Submitting lab	Authors
EPI_ISL_402124	BetaCoV/Wuhan/WIV04/2019	Asia / China / Hubei / Wuhan	2019-12-30	Wuhan Jinyintan Hospital	Wuhan Institute of Virology, Chinese Academy of Sciences	Peng Zhou, Xing-Lou Yang, Ding-Yu Zhang, Lei Zhang, Yan Zhu, Hao-Rui Si, Zhengli Shi
EPI_ISL_406973	BetaCoV/Singapore/1/2020	Asia / Singapore	2020-01-23	Singapore General Hospital	National Public Health Laboratory	Mak, TM; Octavia S; Chavatte JM; Zhou, ZY; Cui, L; Lin, RTP
EPI_ISL_410536	BetaCoV/Singapore/5/2020	Asia / Singapore	2020-02-06	Singapore General Hospital, Molecular Laboratory, Division of Pathology	Programme in Emerging Infectious Diseases, Duke-NUS Medical School	Danielle E Anderson, Martin Linster, Yan Zhuang, Jayanthi Jayakumar, Kian Sing Chan, Lynette LE Oon, Shirin Kalimuddin, Jenny GH Low, Yvonne CF Su, Gavin JD Smith
EPI_ISL_410716	BetaCoV/Singapore/10/2020	Asia / Singapore	2020-02-04	National Public Health Laboratory, National Centre for Infectious Diseases	National Centre for Infectious Diseases, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP
EPI_ISL_410714	BetaCoV/Singapore/8/2020	Asia / Singapore	2020-02-03	National Public Health Laboratory, National Centre for Infectious Diseases	National Public Health Laboratory, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP
EPI_ISL_410715	BetaCoV/Singapore/9/2020	Asia / Singapore	2020-02-04	National Public Health Laboratory, National Centre for Infectious Diseases	National Public Health Laboratory, National Centre for Infectious Diseases	Octavia S, Mak TM, Cui L, Lin RTP